§ 436.217 Film-coat rupture test.

(a) *Immersion fluid.* Dilute 6.0 milliliters of hydrochloric acid to 1,000 milliliters with water. During the performance of the test maintain the immersion fluid at a temperature of 37±0.5 °C by using a thermostatically controlled water bath.

(b) *Immersion vessel.* Use a suitable vessel, such as a 1-liter beaker.

(c) *Operation.* Add 750 milliliters of immersion fluid to the immersion vessel.

(d) *Procedure.* Drop a tablet into the immersion fluid and record the time for the tablet coat to rupture. Repeat the test with a further 19 tablets, testing not more than 10 tablets with a given volume of immersion fluid.

(e) *Evaluation.* The tablets pass the film-coat rupture test if the mean coat rupture time does not exceed 20 seconds and not more than 2 tablets have a coat rupture time exceeding 40 seconds.

[52 FR 42432, Nov. 5, 1987]

Subpart F—Chemical Tests for Specific Antibiotics

§ 436.300 Polarimetric assay of carbenicillin indanyl sodium.

(a) *Equipment.* Polarimeter capable of measuring optical rotatory activity at 365 nanometers: Perkin-Elmer Model 141 or equivalent, with a suitable 1-decimeter polarimeter tube.

(b) *Reagents*—(1) *4-methyl-2-pentanone.* Meets ACS specifications.

(2) *Phosphate-citrate buffer.* Dissolve 61.0 grams of anhydrous disodium phosphate and 11.0 grams of citric acid in 950 milliliters of distilled water. Adjust the pH to 6.0 with 6*N* hydrochloric acid. Dilute to 1,000 milliliters with distilled water.

(c) *Preparation of carbenicillin indanyl sodium sample and working standard solutions.* Accurately weigh approximately 125 milligrams of the carbenicillin indanyl sodium sample or working standard into a 25-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer a 5-milliliter aliquot to a 50-milliliter glass-stoppered centrifuge tube. Add 15 milliliters of the phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone; stopper and shake the tube for 10 seconds. Centrifuge at 2,000 revolutions per minute for 10 minutes to separate the phases. Remove about 15 milliliters of the upper (4-methyl-2-pentanone solvent) phase and proceed as directed in paragraph (e) of this section.

(d) *Preparation of the blank.* Place a 5-milliliter aliquot of distilled water into a 50-milliliter glass-stoppered centrifuge tube, add 15 milliliters of phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone; stopper and shake the tube for 10 seconds. Centrifuge at 2,000 revolutions per minute for 10 minutes to separate the phases. Remove about 15 milliliters of the upper phase and proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Fill the polarimeter tube with the blank solution prepared as described in paragraph (d) of this section. Place the tube in the polarimeter. Adjust the polarimeter to zero rotation using a light source with a wavelength of 365 nanometers. Use the same procedure to determine the optical rotation of both the sample solution and the working standard solution prepared as directed in paragraph (c) of this section.

(f) *Calculations.* Calculate the carbenicillin content (potency) of the sample on an anhydrous basis as follows:

$$\text{Micrograms of carbenicillin per milligram of sample} = \frac{\text{Degrees of rotation of sample solution} \times \text{weight of working standard} \times 100 \times \text{micrograms of carbenicillin in each milligram of the working standard}}{\text{Degrees of rotation of working standard solution} \times \text{weight of sample} \times (100 - m)}$$

where: m=moisture content of the sample.

§ 436.301 Thin layer chromatography identity test for carbenicillin indanyl.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a), (b), (c), and (d) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches lined with Whatman's 3MM chromatographic paper (0.3 millimeters) or equivalent.

(2) *Iodine vapor chamber.* A rectangular tank approximately 9 × 9 × 3.5 inches, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 × 20 centimeters thin layer chromatography plates coated with silica gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Extraction solvent.* Mix ethyl acetate, acetone, pyridine, water, and acetic acid in volumetric proportions of 100:200:25:75:1.5 respectively.

(2) *Developing solvent.* Mix ethyl acetate, acetone, pyridine, water, and acetic acid in volumetric proportions of 300:400:25:75:2 respectively.

(3) *Ferric chloride-potassium ferricyanide reagent.* Immediately before use, mix 100 milliliters of a 1 percent ferric chloride solution in 1 percent hydrochloric acid with 100 milliliters of a 1 percent potassium ferricyanide solution and 75 milliliters of methanol.

(c) *Preparation of working standard solution.* Weigh an amount of the carbenicillin indanyl working standard equivalent to approximately 10 milligrams of carbenicillin into a 50-milliliter Erlenmeyer flask. Dissolve the material in sufficient extraction solvent to make a solution containing 1 milligram carbenicillin per milliliter.

(d) *Procedure.* Pour developing solvent into the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 2 centimeters, spot 10 microliters of the standard solution and the sample solution. The plate should be air dried for 30 minutes. Place the plate into the chromatography tank. Allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Heat the plate for 30 minutes at 80° C. in a circulating air oven and then allow the plate to cool to room temperature. Place the plate in the iodine vapor chamber for about 30 seconds, remove the plate and spray it with the ferric chloride-potassium ferricyanide reagent. Carbenicillin indanyl appears as a blue spot on a yellow-green background at an R_f of about 0.5. The test is satisfactory if the sample compares qualitatively with the standard.

[39 FR 18944, May 30, 1974, as amended at 41 FR 18509, May 5, 1976]

§ 436.302 Clindamycin vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector: Barber-Colman 5,000 or equivalent.

(b) *Reagents.* (1) Pyridine, reagent grade, dried over sodium sulfate.

(2) Chloroform, reagent grade.

(3) Acetic anhydride, reagent grade, used as acteylating agent.

(4) Internal standard: Prepare a solution containing 3 milligrams of cholestane per milliliter in pyridine.

(c) *Typical conditions.* (1) Column: 4 feet × 4 millimeters ID, glass, with 1 percent SE–30 on Diatoport S (60/80 mesh), or equivalent.

(2) Temperatures: Column 200° C.; detector 215° C.; injection port, ambient temperature.

(3) Carrier gas: Helium approximately 120 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen at 120 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 2 for clindamycin, 1 for internal standard: 2×10^{-8} amperes.

(d) *Preparation of clindamycin sample and working standard solutions.* Accurately weigh approximately 15 milligrams of sample or working standard into a glass-stoppered conical 15-milliliter centrifuge tube. Add 1.0 milliliter of chloroform, 1.0 milliliter of internal standard solution, and 0.6 milliliter of acetic anhydride. Agitate the tubes to insure dissolution of the sample and